

### Clinical Study Protocol

Study Number and Protocol Title	EM-05-013953 96-hour Antimicrobial persistence assessment [REDACTED] [REDACTED] Following Exposure to Saline and Blotting
Principal Investigator	[REDACTED]
Sub-Investigators:	[REDACTED] [REDACTED]
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IRB/EC	Name: Chesapeake Institutional Review Board Address: 6940 Columbia Gateway Drive, Suite 110, Columbia, Maryland, 21046, USA

[REDACTED]

Sponsor	3M Health Care [REDACTED] 3M Center [REDACTED] St. Paul, MN 55144-1000
3M Study Monitor	[REDACTED]
3M Medical Monitor	[REDACTED]

[REDACTED]		
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]		[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

**3M**  
Health Care  
3M Center  
St. Paul, MN 55144-1000

Page 3 of 44  
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## Table of Contents

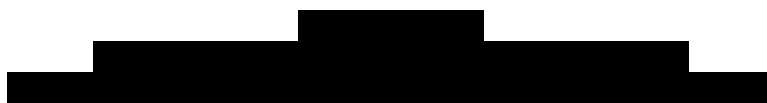
<b>1. BACKGROUND INFORMATION .....</b>	<b>7</b>
1.1 Name, Description and Intended Use of the Investigational Product .....	7
1.2 Summary of Relevant Studies .....	7
1.3 Risk/Benefit Summary .....	8
1.4 Investigational Material Application .....	8
1.5 Good Clinical Practices and Regulatory Requirements .....	8
1.6 Study Population .....	8
<b>2. STUDY OBJECTIVES .....</b>	<b>9</b>
<b>3. STUDY DESIGN .....</b>	<b>9</b>
3.1 Study Type and Methodology .....	9
3.2 Primary and Secondary Endpoints .....	12
3.3 Randomization and Blinding .....	12
3.4 Study Products and Labeling .....	12
3.4.1 Study Products .....	12
3.4.2 Investigational Products Labeling and Accountability .....	13
3.4.3 Other Product Labeling .....	13
3.4.4 Additional Study Supplies .....	14
3.5 Study Duration .....	15
3.6 Study Termination/Subject Discontinuation or Withdrawal .....	15
3.6.1 Study Termination .....	15
3.6.2 Subject Discontinuation or Withdrawal .....	16
3.7 Source Data .....	16
3.8 Computerized Systems .....	16
3.9 Protocol Modifications .....	16
3.9.1 Protocol Amendments .....	16
3.9.2 Protocol Deviations .....	17
<b>4. SUBJECT SELECTION .....</b>	<b>17</b>
4.1 Subject Inclusion Criteria .....	17
4.2 Subject Exclusion Criteria .....	18
4.3 Subject Informed Consent .....	19
<b>5. TREATMENT OF SUBJECTS .....</b>	<b>19</b>
5.1 Medication(s)/Treatment(s) Not Permitted .....	19
5.2 Subject Compliance .....	19
5.3 Study Procedures .....	19
5.3.1 Pretreatment Phase (Washout) .....	19
5.3.2 Screening Phase .....	20
5.3.3 Treatment Phase .....	20
<b>6. ASSESSMENT OF EFFICACY .....</b>	<b>22</b>



6.1	Efficacy Parameters.....	22
6.2	Assessment Methods .....	23
6.2.1	Microbiological Methods .....	23
6.3	Subject Discontinuation.....	23
<b>7.</b>	<b>ASSESSMENT OF SAFETY .....</b>	<b>24</b>
7.1	Safety Parameters .....	24
7.2	Assessment Methods .....	24
7.3	Adverse Events.....	24
<b>8.</b>	<b>STATISTICS .....</b>	<b>25</b>
8.1	Data Sets Analyzed.....	25
8.2	Statistical Methods .....	25
8.2.1	Efficacy Analyses .....	25
8.2.2	Safety Analyses .....	25
8.3	Sample Size Justification .....	26
8.4	Criteria for Termination of the Study .....	26
8.5	Procedures for Accounting for Missing, Unused, and Spurious Data.....	26
8.6	Deviations to Statistical Plan .....	26
<b>9.</b>	<b>MONITORING .....</b>	<b>26</b>
<b>10.</b>	<b>QUALITY CONTROL AND QUALITY ASSURANCE.....</b>	<b>26</b>
<b>11.</b>	<b>ETHICS .....</b>	<b>26</b>
<b>12.</b>	<b>DATA HANDLING AND RECORD KEEPING .....</b>	<b>27</b>
12.1	Study Personnel.....	27
12.2	Pre-Study Documentation Requirements .....	27
12.3	Completion and Return of Case Report Forms .....	27
12.4	Final Report.....	27
12.5	Records, Reports and Retention Requirements.....	27
<b>13.</b>	<b>REFERENCES .....</b>	<b>28</b>
<b>14.</b>	<b>APPENDICES .....</b>	<b>29</b>
14.1	Study Flow Diagrams .....	29
14.2	Study Product Application Instructions.....	31
14.3	Confirmation of Release & Receipt of Clinical Supplies .....	32
14.4	Study Product Disposition Form.....	33
14.5	Subject Instructions .....	34
14.6	Elements of Informed Consent.....	35
14.7	Test Site Diagrams .....	37
14.8	Skin Irritation Rating Scale.....	38
14.9	Protocol for Neutralization Validation .....	39
14.9.1	Background .....	39
14.9.2	Objective .....	39



14.9.3 Subject Entry Criteria .....	39
14.9.4 Test Organism .....	40
14.9.5 Study Products .....	40
14.9.6 Materials, Supplies and Equipment .....	41
14.9.7 Study Procedures .....	42
14.9.8 Control of Bias .....	43
14.9.9 Data Evaluation .....	43



## 1. BACKGROUND INFORMATION

[REDACTED]

The purpose of this study is to evaluate [REDACTED] 3M CHG/IPA Prep compared with a commercially available skin prep [REDACTED] in suppressing the growth of residual skin flora by measuring the regrowth of normal skin flora following exposure to fluids and mechanical removal by wiping on the abdomen at 48 hours, 72 hours and 96 hours.

### 1.1 Name, Description and Intended Use of the Investigational Product

[REDACTED] (3M CHG/IPA Prep) is an investigational new drug containing 2% w/v chlorhexidine gluconate (CHG) and 70% v/v isopropyl alcohol (IPA) [REDACTED]

[REDACTED] contains 2% w/v CHG and 70% v/v IPA and will be used as the marketed comparator prep. [REDACTED]

### 1.2 Summary of Relevant Studies

[REDACTED]  
[REDACTED] Refer to the Investigator Brochure [REDACTED]  
for a summary of these studies.

[REDACTED]

### 1.3 Risk/Benefit Summary

There are minimal anticipated adverse health risks for the participants of this study. Considerable safety and efficacy data are available for the active ingredients in the investigational product and preclinical safety studies. [REDACTED], have been completed.

[REDACTED]

There are no direct health benefits to subjects who participate in this study.

### 1.4 Investigational Material Application

This study will be conducted using [REDACTED] 3M CHG/IPA Prep and [REDACTED]. Both products will be applied topically to intact skin of the subject's abdominal and inguinal regions.

The products will be applied as intended per instructions for use [REDACTED]

[REDACTED]

### 1.5 Good Clinical Practices and Regulatory Requirements

[REDACTED]

[REDACTED]

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), Declaration of Helsinki and applicable state, federal and regional regulations including 21 CFR 312 (Investigational New Drug Application), 50 (Protection of Human Subjects), 56 (Institutional Review Boards), 54 (Financial Disclosure by Clinical Investigators). And in accordance with the European Directive 95/46/EC on the protection of individuals with regard to the processing of personal data and on the free movement of such data and with the Romanian Order No 677/2001 for the protection of persons concerning the processing of personal data and free circulation of such data. And in spirit with Directive of the European Parliament and Council 2001/20/EC, and Romanian Order No. 904/25.07.2006.

### 1.6 Study Population

Healthy subjects, ages 18 or older, of either gender who meet the study inclusion/exclusion criteria are eligible for enrollment into this study.

A sufficient number of subjects will be enrolled in the screening phase to provide a minimum of 50 treatment sites per anatomical region, per treatment, per collection time that meet the baseline requirements on treatment day and have an intact dressing at the collection time. Subjects must satisfy all Screening Day and Treatment Day Inclusion/Exclusion Criteria prior to collection of screening baselines and Treatment Day procedures. If the required numbers of subjects do not

[REDACTED]

qualify from the initial screening group, additional subjects will be recruited, as needed. Treatment day baseline requirement is greater than or equal to  $3.0 \log_{10}$  CFU/cm<sup>2</sup> on the abdomen and greater than or equal to 5.0 CFU/cm<sup>2</sup> on the groin.

Each subject will be identified by a screening identification number, and a treatment number. Subject treatment numbers will not be assigned until a subject has passed the screening criteria, including screening baseline bacterial counts.

Both the right and left sides of the abdomen and/or groin must meet the screening baseline requirements stated in the Inclusion Criteria to qualify for treatment of that anatomical region in the study.

## **2. STUDY OBJECTIVES**

The primary objective of this study is to evaluate the persistent antimicrobial effectiveness of the 3M CHG/IPA Prep ( ) and ( ) on the abdomen and groin following a saline and wipe challenge by measuring the regrowth of normal skin flora at 48 hours, 72 hours and 96 hours. The secondary objective is to evaluate the suppression of regrowth relative to post-prep (10-minute) of 3M CHG/IPA Prep and ( ) at 48 hours, 72 hours and at 96 hours post-prep.

Safety will also be evaluated based on the incidence of adverse events reported during the study and assessment of skin irritation ratings.

## **3. STUDY DESIGN**

### **3.1 Study Type and Methodology**

This is a randomized, controlled, third-party blind, single-center study in healthy subjects using a paired comparison design where each subject receives both study products on the abdomen and/or groin. The study staff performing the bacterial enumeration and the statistician performing the analysis will be blinded to the study products.




This study will be conducted using a modification of ASTM E1874-14 Standard Test Method for Recovery of Microorganisms from Skin using the Cup Scrub Technique.<sup>1</sup>

Time points for sample collection are baseline, 10 minutes, 48 hours, 72 hours and 96 hours. After collection of the 10 minute time point sample, a repetitive saline soak and wiping procedure is performed to simulate the fluid and mechanical removal challenges occurring in surgery.

Table 3.1A provides a summary of the study procedures. A schematic diagram of the study is included in Appendix 14.1.



**Table 3.1A Study Summary**

14-day Pretreatment Phase	Screening Phase	Treatment Phase			
		T=0	T=48 hours	T=72 hours	T=96 hours
Perform the Informed Consent process. The Inclusion/Exclusion Criteria will be reviewed to ensure eligibility for the study. If these criteria are satisfied, subjects will sign the consent form.	Complete Inclusion/Exclusion Criteria form;	Assign sequential subject number; Complete Inclusion/Exclusion Criteria form	Complete 48-hour Compliance Form	Complete 72-hour Compliance Form	Complete 96-hour Compliance Form
Assign subject screening number	Perform visual skin assessment	Perform visual skin assessment	Perform skin irritation rating	Perform skin irritation rating	Perform skin irritation rating
Complete Inclusion/Exclusion Criteria form	Collect screening baseline samples from abdominal and inguinal regions	Perform skin irritation rating	Collect 48-hour  post-prep regrowth samples	Collect 72-hour  post-prep regrowth samples	Collect 96-hour  post-prep regrowth samples
Review subject instructions for wash out and dispense subject personal hygiene kit	Count screening plates, determine which subjects qualify for study	Per randomization, mark test areas, collect baseline samples from abdominal and inguinal regions	Check the dressings covering the 72-hour and 96-hour sampling sites and reinforce as necessary.	Check the dressings covering the 96-hour sampling sites and reinforce as necessary.	



Schedule for clipping 72 hours prior to screening, if needed.	Contact eligible subjects, schedule Treatment Day (schedule for clipping 72 hours prior to treatment, if needed)	Per randomization, apply study products			
		Perform skin irritation rating, collect 10-minutes [REDACTED] post-prep samples			
		Challenge test sites with repetitive saline soak and wiping: [REDACTED]			
		[REDACTED] 48-, 72- and 96-hour regrowth sites with semi-occlusive dressings			

[REDACTED]

### 3.2 Primary and Secondary Endpoints

#### Primary Endpoint:

The primary measure of persistence is the suppression of regrowth relative to baseline of 3M CHG/IPA Prep and [REDACTED] at 48 hours, 72 hours and at 96 hours post-prep.

#### Secondary Endpoint:

The secondary measure of persistence is the suppression of regrowth relative to post-prep (10-minute) of 3M CHG/IPA Prep and [REDACTED] at 48 hours, 72 hours and at 96 hours post-prep.

The principal measure of safety will be the incidence of adverse events reported during the study and a summary of the skin irritation rating scores. [REDACTED]

### 3.3 Randomization and Blinding

The left and right test areas on each subject's abdominal and inguinal regions will be randomized to receive either the investigational product, 3M CHG/IPA Prep, or the marketed comparator product, [REDACTED]. The inguinal region baseline and all post-prep samplings will be randomized to sites within each test area. The Investigator is responsible to assure that the study randomization is followed. The randomization schedule will be provided to the study site in a separate document.

The study products cannot be blinded from the Investigator or study staff performing the product application, sample collection and skin assessment due to the obvious differences in applicator design and other physical characteristics. However, the study staff performing the bacterial enumeration will not be involved in the study product application or the collection of samples and, therefore, will be blinded to the study products. The statistician performing the analysis will also be blinded until after data lock.

### 3.4 Study Products and Labeling

#### 3.4.1 Study Products

- Investigational prep: 3M™ CHG/IPA Film-forming Patient Preoperative Skin Preparation, 2% w/v CHG/70% v/v IPA. [REDACTED]
- Comparator prep: [REDACTED]  
2% w/v CHG/70% v/v IPA [REDACTED]

3M CHG/IPA Prep and the marketed comparator prep will be applied topically to intact skin of the abdominal and inguinal (groin) regions of the subject. The prep will be applied with repeated back-and-forth strokes for 30 seconds on the abdomen or 2 minutes on the groin and allowed to dry for 3 minutes.

[REDACTED]

Detailed instructions for application and removal of the study products are provided in the Investigational Product Application Instructions (Appendix 14.2).

### 3.4.2 Investigational Products Labeling and Accountability

3M will label, package and ship the study products to the research facility. The 3M CHG/IPA Prep will be provided in individual [REDACTED] packages, and labeled as below.

#### Sample Investigational Product Label

**CAUTION: New Drug-Limited by Federal Law to Investigational Use**  
For use in study EM 05-013953  
3M Health Care, St. Paul, MN 55144-1000  
  
3M CHG/IPA Prep [REDACTED] **Subject Number:**  
Product ID: [REDACTED]  
Lot: TBD  
  
Directions: Use as directed in protocol  
**Warning: Contents are flammable until dry.**

3M requires Investigators to maintain accountability and adequate inventory security of the study products at all times. The Investigator or designee will:

- Document receipt of study products on the Confirmation of Release & Receipt of Clinical Supplies form (Appendix 14.3) and return the completed form to 3M,
- Store study products in a secure facility accessible only to authorized individuals,
- Dispense study products only to subjects properly enrolled into the study,
- Account for inventory and disposition using the Study Product Disposition Form (Appendix 14.4), and
- Dispose of used and unused study products as agreed upon. Once accounted for the study products may be disposed. Documentation shall be maintained concerning the disposal and will contain: the quantity of the study product(s) disposed of, the date and manner of disposal, the staff member who conducted the disposal. NOTE: Record the subject number on the package label and retain the label for inventory purposes. The used applicator can be discarded.

In addition, copies of all completed Study Product Disposition Forms and disposal records must be retained in the Investigator Study Documentation File. The originals of all Study Product Disposition Forms and disposal records must be sent to the Sponsor's Clinical Monitor.

### 3.4.3 Other Product Labeling

The comparator prep, [REDACTED] is available for commercial use. The [REDACTED] products will be provided in original marketed packaging with an over label, as below, placed on each applicator pouch.



Sample Marketed Comparator Prep Label

***Commercialized Product***

For use in study EM-05-013953

3M Health Care, St. Paul, MN 55144-1000



**Subject Number:**

Directions: Use as directed in protocol

**Warning: Contents are flammable until dry.**

**3.4.4 Additional Study Supplies**

Sponsor will provide the following:

- Study Material Disposition forms
- Randomization schedule
- Data Collection Sheets (DCSs)
- Investigational products



Study site will provide the following:

- Subject Informed Consent forms, IRB/EC-approved





- The discovery of an unexpected, serious, or unreasonable risk to study subjects,
- Insufficient adherence to protocol requirements,
- Failure of the Investigator to enroll subjects into the study at an acceptable rate,
- Failure of the Investigator to comply with pertinent state, federal or regional regulations or applicable privacy regulations,
- Submission of knowingly false information from the Investigator to 3M,
- Termination of IND or IRB/EC approval, or withdrawal of the IRB/EC approval,
- A decision on the part of 3M to suspend or discontinue evaluation of the investigational product.

### **3.6.2 Subject Discontinuation or Withdrawal**

The Investigator may discontinue individual subjects from the study at any time. Subjects entering the study may withdraw at any time without penalty. The right of each test subject to withdraw from the study and the right of each test subject to privacy, confidential treatment of personal data, and protection from view by others not conducting the study will be respected at all times. The Investigator will provide a written report on the appropriate case report form including the date and reason for discontinuance.

### **3.7 Source Data**

Electronic data capture (eDC) will be used for this study. If not entered directly into eDC, data will first be recorded onto a DCS, followed by entry into the appropriate electronic case report form (eCRF). Wherever the data are entered first is considered the source document.

### **3.8 Computerized Systems**

All study data will be loaded into the [REDACTED] data management system.

The current 3M Health Care document storage system, [REDACTED], will be used to store the study documents.

[REDACTED], may be used for study documentation and data analysis.

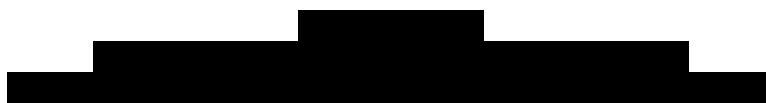
### **3.9 Protocol Modifications**

#### **3.9.1 Protocol Amendments**

A protocol amendment is defined as a change in procedure that is identified and documented before initiation. A significant amendment means one that affects the safety, rights or welfare of subjects, the scope of the investigation or the scientific quality of the study.

All protocol amendments must be documented and approved by the Investigator and study monitor prior to implementation. All protocol amendments must also be pre-approved by the reviewing IRB/EC prior to implementation.

3M will submit significant protocol amendments to the Investigator for submission to the IRB/EC. 3M will also notify the Investigator when a protocol amendment may be implemented.



### 3.9.2 Protocol Deviations

A protocol deviation is defined as a change in procedure that has not been documented before initiation and therefore has not been reviewed and approved with the study sponsor and/or reviewing IRB/EC prior to initiation. All deviations from the study protocol are to be documented and reported to the investigator/study sponsor and reviewing IRB/EC.

A deviation is a departure from the protocol that will likely affect the safety, rights or welfare of subjects, the scope of the investigation or the scientific quality of the study.

A protocol deviation is only for an individual subject. Deviations that potentially affect 1) subject safety, rights or welfare, 2) data integrity or 3) compromise the statistical analysis of the study require immediate communication to 3M. The Investigator must contact the 3M study monitor [REDACTED]. A Protocol Deviation Form must be completed by the Investigator and include the type of deviation and a description of the circumstances surrounding the deviation. A copy is sent to the 3M study monitor [REDACTED] identifying the occurrence.

Deviations, which are made to protect the life or physical well-being of a subject in an emergency, must be reported by the Investigator to the IRB/EC within 5 working days of occurrence.

## 4. SUBJECT SELECTION

Healthy subjects will be recruited. A suitable number of subjects will be enrolled in the pretreatment, screening and treatment phase, such that a total of 50 subjects are evaluable for efficacy at completion of the study.

Subjects must follow all Subject Instructions (Appendix 14.5) and satisfy inclusion/exclusion criteria prior to enrollment into the treatment phase of the study.

All subjects will be provided verbal and written information about the study procedures, and subject instructions (Appendix 14.5). The following inclusion/exclusion criteria will be reviewed on pretreatment, screening and treatment day to establish eligibility for participation:

### 4.1 Subject Inclusion Criteria

Subjects to whom *all* of these conditions apply will be eligible for enrollment in this study:

1. Subjects of either gender and any race who are at least 18 years of age,
2. Subjects must be in good general health,
3. Subjects who agree to be and appear to be compliant with the requirements of the study,
4. Subjects who have good skin condition on the test sites,
5. Subjects who are willing to report to the study facility approximately 72 hours prior to Screening or Treatment Day for clipping, if needed,
6. Subjects who are willing to avoid showering and tub-bathing within 72 hours prior to Screening and Treatment Days (sponge-baths may be taken; however, the lower abdomen and upper thigh regions must be avoided),



7. Subjects who are willing to avoid showering, tub-bathing, swimming, and vigorous physical activity that may cause sweating once study products have been applied,
8. Subjects who are willing to return to the study facility at 48 hours, 72 hours and 96 hours after treatment for sample collection, and
9. Subjects who have Screening Day baseline counts of  $\geq 3.00 \log_{10}$  per  $\text{cm}^2$  bilaterally on the abdominal region and/or  $\geq 5.00 \log_{10}$  per  $\text{cm}^2$  bilaterally on the inguinal region.

#### **4.2 Subject Exclusion Criteria**

Subjects to whom *any* of these conditions apply will be excluded from this study:

1. Participation in another clinical study in the past 30 days, current participation in another clinical study, or previous participation in this study,
2. Has taken antihistamines in the 48 hours prior to Treatment Day,
3. Any tattoos, scars, breaks in the skin, or any form of dermatitis, or other skin disorders (including acne) on the applicable test areas,
4. A history of skin allergies,
5. A history of skin cancer within 6 inches of the test areas,
6. Known sensitivity to acrylate-, chlorhexidine gluconate-, or alcohol-containing products, or to medical tape, metals, natural rubber latex, vinyl, or skin-marking inks,
7. A medical diagnosis with physical condition that may put the subject at risk, such as a current or recent severe illness, hepatitis, organ transplant, congestive heart disease, or any immunocompromised conditions, such as AIDS (or HIV positive),
8. Any medical condition or use of any medications that, in the opinion of the Investigator, should preclude participation,
9. Pregnancy, possible pregnancy, attempting pregnancy, or nursing,
10. Topical antimicrobial exposure within 14 days prior to Screening Day and throughout the study. Restrictions include, but are not limited to antimicrobial soaps, medicated shampoos, medicated lotions, antiperspirants/deodorants, perfumes, after shaves, and colognes,
11. Use of systemic or topical antibiotic medications, steroid medications (other than for hormonal contraception or postmenopausal reasons), or any other product known to affect the normal microbial flora of the skin within 14 days prior to Screening Day and throughout the study,
12. Exposure of the test areas to solvents, acids, bases, strong detergents, fabric softener-treated clothing, or other household chemicals within 14 days prior to Screening Day and throughout the study,
13. Swimming in chemically treated pools or bathing in hot tubs, spas, or whirlpools within 14 days prior to Screening Day and throughout the study,
14. Use of tanning beds, hot waxes, or depilatories (in the applicable test areas) within 14 days prior to Screening Day and throughout the study
15. Bathing and showering within 72 hours prior to Screening Day and throughout the study, or



16. Subject has used moisturizers or any topical treatment (eg lotion, sunscreen or shaving cream) on the test sites in the 24-hour period prior to participation in the study.

#### **4.3 Subject Informed Consent**

The Investigator or designee must ensure that written informed consent to participate in the investigation is obtained before including any individual as a subject in the investigation. The Investigator or designee must provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate, and minimize the possibility of coercion or undue influence. The process is designed to 1) give the subject all the information that they need, 2) ensure that the subject understands the information and 3) give the subject a chance to consider study participation. The process should permit the subject to ask questions and exchange information freely.

Specifically, the Investigator or designee is to explain to each subject all elements of informed consent as specified in 21 CFR 50.25 (Appendix 14.6). After the explanation, the subject or representative will voluntarily sign and date the consent form if they wish to participate in the study. A copy of the consent form must be provided to the subject. A signed and dated copy of the consent form must be maintained in the Investigator study documentation file at all times. Consent and study participation, with date, must be documented in the subject's record.

### **5. TREATMENT OF SUBJECTS**

#### **5.1 Medication(s)/Treatment(s) Not Permitted**

Exposure of the test areas to antimicrobial agents is not permitted within 14 days prior to Screening Day and for the duration of the study. Restrictions include, but are not limited to antimicrobial soaps, antiperspirants/deodorants, shampoos, lotions, perfumes, after shaves, colognes, steroid medications (other than for hormonal contraception or postmenopausal reasons), and topical or systemic antibiotics.

#### **5.2 Subject Compliance**

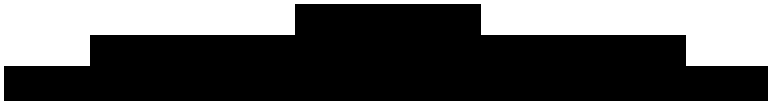
Answers to the Inclusion/Exclusion Criteria questions asked at the beginning of the Screening and Treatment Phases, and Compliance questions prior to the 48 hour, 72 hour and 96 hour sample collection will determine compliance to the requirements of the study.

#### **5.3 Study Procedures**

These study procedures are based on ASTM E1173-15, Standard Test Method for Evaluation of Preoperative, Precatheterization, or Preinjection Skin Preparations.<sup>2</sup>

##### **5.3.1 Pretreatment Phase (Washout)**

The Inclusion/Exclusion Criteria will be reviewed with each subject to ensure eligibility for the study. If these criteria are satisfied, subjects will sign the consent form. Prior to the scheduled Screening Day, subjects will undergo a minimum 14-day pretreatment phase to allow for the removal of any antimicrobial agents from the subjects' skin. Subjects are to refrain from the use of products containing antibacterial agents as written in the Subject Instructions (Appendix 14.5). Subjects will be given personal hygiene kits containing non-antimicrobial products for use



during this period. Subjects will be instructed to use these products through completion of the Treatment Phase or until notified by the Investigator or designee.

In addition, subjects must avoid systemic or topical antibiotics or medications and contact with chemically treated swimming pools or hot tubs, hot waxes, and depilatories on the test areas for 14-days before the scheduled Screening Day. If it becomes necessary to take systemic antibiotics or to apply topical medications to the test areas within this pretreatment period, the subject must contact the Investigator as soon as reasonably possible so that another subject may be recruited.

If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility approximately 72 hours before the Screening Day. Subjects will not be allowed to shower or bathe for 72 hours prior to their scheduled screening appointment. Sponge-baths may be taken; however, the lower abdomen and upper thigh regions must be avoided.

### **5.3.2 Screening Phase**

Prior to collection of the screening baseline samples, the Investigator or a designee will complete the screening Inclusion/Exclusion form. A visual skin assessment of the test areas will be performed and the screening baseline samples will be collected using the cup scrub technique described in Section 6.2.1.1. [REDACTED]

Samples from both the left and right sides of the abdominal and/or inguinal regions must meet the values indicated in the Inclusion Criteria to be enrolled into the Treatment Phase of the study. Those subjects who qualify will be notified and will continue to follow the instructions in Appendix 14.5 until completion of the scheduled Treatment Day. If subjects require hair removal to facilitate sample collection, the subject will be asked to return to the test facility approximately 72 hours before Treatment Day. Subjects will not be allowed to shower or bathe for 72 hours prior to Treatment Day. Sponge-baths may be taken; however, the lower abdomen and upper thigh regions must be avoided.

### **5.3.3 Treatment Phase**

A sufficient number of subjects who meet the Inclusion Criteria will be enrolled into the Treatment Phase of the study, such that at least 50 abdominal and 50 inguinal regions (randomized between left and right sides) are evaluable at completion of the study.

The Investigator or a designee will complete the treatment Inclusion/Exclusion form. If these criteria are satisfied, a visual skin assessment will be performed to evaluate the condition of each test area.

#### **5.3.3.1 Preparation of Test Areas**

A Test Site Diagram for the abdominal test areas is shown in Appendix 14.7.

##### Abdominal Region:

The test area within the abdominal region is defined as the area below and to the right or left of the umbilicus, approximately midway between the umbilicus and the pubis. Using a 6" x 6" sterile template, the corners of each abdominal test area will be marked directly onto the skin



using a non-toxic skin marker. Five sampling sites will be numbered within each abdominal test area. The positioning and numbering of the abdominal sampling sites are standard for all subjects [REDACTED]

[REDACTED] The 5 sampling sites within each abdominal test area represent the baseline (pre-prep) site, 4 post-prep sample sites (10-min, 48-hr, 72-hr, 96-hr). Sampling sites [REDACTED] will be randomized as the 4 post-prep sample sites. Sampling site [REDACTED] is designated as the baseline sample site.

After abdominal test areas are marked and sampling sites are numbered, baseline samples will be collected from the right and left test areas [REDACTED] using the cup scrub technique described in Section 6.2.1.1.

### Inguinal Region

The test area within the inguinal region is defined as the uppermost inner aspect of the thigh (the inguen), centering the long axis of the template along the inguinal crease immediately below the groin. If, due to a subject's anatomy, the test area cannot be centered along the inguinal crease, the area should be positioned on the upper, inner thigh as close to the crease as possible. In no instance should sampling be performed on areas not having skin-to-skin contact. Using a 2" x 6" sterile template, the corners of each inguinal test area will be marked directly on the skin using a non-toxic skin marker. Five sampling sites will be numbered within each inguinal test area. The positioning and numbering of the inguinal sampling sites are standard for all subjects. [REDACTED]

[REDACTED] The 5 sampling sites within each inguinal test area represent one baseline (pre-prep) site and the 4 post-prep sample sites (10-min, 48-hr, 72-hr and 96-hr). Sites [REDACTED] will be randomized as the baseline and 10-min sampling sites. Sites [REDACTED] will be randomized as the 48-hr, 72-hr and 96-hr sampling sites.

After inguinal test areas are marked and sample sites are numbered, baseline samples will be collected from the right and left test areas per the randomization schedule using the cup scrub technique described in Section 6.2.1.1.

### **5.3.3.2 Study Product Application**

Following baseline sample collection, contralateral abdominal and/or inguinal test areas will each be prepped with 1 of the 2 study products according to the randomization provided. Treatments will be randomized between left and right test areas.

The study product will be applied per the randomization schedule and the Investigational Product Application Instructions (Appendix 14.2). The duration of each prep procedure will be recorded on the appropriate form.

### **5.3.3.3 Evaluation of Skin Irritation**

Prior to collection of the baseline and post-prep samples (10-min, 48-hour, 72-hour and 96-hour), the skin in each test area will be evaluated for indications of skin irritation, based on the Skin Irritation Rating Scale (Appendix 14.8). Skin irritation ratings for each area will be recorded on the Skin Irritation Rating form. [REDACTED]

[REDACTED]

[REDACTED]  
Any area with a rating of 3 should not be sampled.

#### **5.3.3.4 Timing of Post-Prep Sample Collection**

Microbial samples will be collected at 10 minutes [REDACTED], 48 hours [REDACTED] 72 hours [REDACTED] and 96 hours [REDACTED] post-prep from the abdomen and/or groin. Post-prep timing begins upon completing the application of the study products, including the 3-minute drying time. Microbial samples will be collected using the cup scrub technique described in Section 6.2.1.1.

##### **5.3.3.4.1 Saline and Wiping Challenge**

After the 10-minute post-prep samples have been collected, the remaining sample sites (48 hour, 72 hour and 96 hour) will be challenged with repetitive saline soaks and blotting [REDACTED]

[REDACTED]  
After the repetitive saline soak and wiping challenge is complete, a sterile semi-occlusive dressing will be secured over the challenged sample sites (48 hour, 72 hour and 96 hour) to allow subjects restricted mobility and to protect the sites from contamination between sampling times.

[REDACTED] The subjects will be allowed to leave the clinical test facility as long as they return 48 hours, 72 hours and 96 hours later for collection of the 48-hour, 72-hour and 96-hour samples. Subjects should be reminded that they are to avoid showering, tub-bathing, swimming, vigorous physical activity that may cause sweating, and any other activities that may compromise the integrity of the test sites until their final sample collection has been completed.

In the event that the dressings on the 48-hour, 72-hour and/or 96-hour sampling sites have been compromised, the site will not be sampled and the loss of dressing integrity will be recorded on the appropriate data collection sheet (DCS).

The dressings covering the 72-hour and 96-hour sampling sites will be observed and reinforced as necessary during the 48-hour post-prep time point. The dressings covering the 96-hour sampling sites will be observed and reinforced as necessary during the 72-hour post-prep time point.

Following the final sample collection, residual study products will be removed from the subject's skin [REDACTED].

## **6. ASSESSMENT OF EFFICACY**

### **6.1 Efficacy Parameters**

The primary measure of persistence is the suppression of regrowth relative to baseline of 3M CHG/IPA Prep and [REDACTED] at 48 hours 72 hours and at 96 hours post-prep.

[REDACTED]

The secondary measure of persistence is the suppression of regrowth relative to post-prep (10-minute) of 3M CHG/IPA Prep and [REDACTED] at 48 hours 72 hours and at 96 hours post-prep.

## **6.2 Assessment Methods**

### **6.2.1 Microbiological Methods**

#### **6.2.1.1 Sample Collection and Processing**

Quantitative cultures will be obtained from sampling sites using a modification of the cup scrub method of Williamson and Kligman.<sup>3</sup>

[REDACTED]

A sterile scrub cup will be placed and held firmly on each site. **Scrub 1:** [REDACTED] SSS will be aseptically pipetted into the scrub cup. The skin will be scrubbed in a circular motion with moderate pressure for 1 minute using a sterile policeman. Using a sterile transfer pipette, the scrub solution will be transferred to a sterile collection tube. **Scrub 2:** [REDACTED] fresh SSS will be pipetted into the scrub cup and the scrub procedure will be repeated. This solution will be pooled with the solution from Scrub 1 for a total sample volume of approximately 6 mL per site.

Following collection, each sample tube will be capped tightly [REDACTED] followed by a [REDACTED] 30-second vortex. A 1.0 mL aliquot of each sample will be diluted into sterile tubes containing 9.0 mL PBW. Serial 10-fold dilutions will be performed in PBW. One-milliliter (1.0-mL) aliquots of selected dilutions will be pour-plated in duplicate using TSA+N. **Samples must be plated within 30 minutes of collection.** [REDACTED] After 72 [REDACTED] hours of aerobic incubation at  $30 \pm 2^{\circ}\text{C}$ , colonies will be counted and viable cells in the original sample will be calculated using standard methods. [REDACTED]

#### **6.2.1.2 Collection of Raw Data**

Raw colony counts from each of the dilutions will be recorded on the appropriate data collection sheet (DCS) for each subject.

#### **6.2.1.3 Neutralization of Investigational Products**

Prior to Treatment Day the effectiveness of the neutralizers contained in the microbial sampling solution will be tested based on ASTM E1054-08 (2013), Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents.<sup>4</sup> The protocol for neutralization validation is provided in Appendix 14.9.

## **6.3 Subject Discontinuation**

The Investigator or designee may discontinue individual subjects from the study at any time. Subjects may voluntarily withdraw from the study at any time. The Investigator or designee will

[REDACTED]

provide a written report on the appropriate form including the date and reason for discontinuation. Subjects who have been previously screened may not be re-entered into the study.

## **7. ASSESSMENT OF SAFETY**

### **7.1 Safety Parameters**

The principal measure of safety will be the incidence of adverse events reported during the study and a summary of the skin irritation rating scores. [REDACTED]

### **7.2 Assessment Methods**

The Investigator or designee will assess the subject's skin condition prior to collection of the baseline, 10-min, 48-hour, 72-hour, and 96-hour post-prep samples using the Skin Irritation Rating Scale (Appendix 14.8).

### **7.3 Adverse Events**

The Investigator is responsible for identifying adverse events that occur to each subject. An adverse event can be identified by the Investigator or reported by the subject.

#### **Definitions:**

- Adverse event (AE) means any undesirable clinical occurrence in a subject whether or not it is considered to be drug related.
- Drug-related adverse event (ie, adverse drug experience) is an adverse event that is considered by the Investigator to have a reasonable likelihood of being associated with the investigational drug.
- Life-threatening adverse drug experience is any adverse drug experience that places the subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred (does not include a reaction that, had it occurred in a more severe form, might have caused death).
- Serious adverse drug experience is an adverse drug experience that is fatal or life-threatening, is permanently or significantly disabling/incapacitating, requires inpatient hospitalization or prolongation of existing hospitalization, or results in a congenital anomaly/birth defect.
- Unexpected adverse drug experience is one not previously identified in nature, severity or frequency of incidence in the current Investigator brochure, in the clinical plan, or elsewhere in the current IND application and amendments.

#### **Recording and Reporting:**

The Investigator records each AE occurring in the study, including those not thought to be associated with the drug, on the Adverse Event Record. Documentation includes the AE description, severity at onset, seriousness, date of onset and resolution, relationship to the investigational drug, action taken, and outcome.

[REDACTED]

The Investigator must promptly report all adverse drug experiences to the 3M study monitor. If the adverse drug experience is also considered by the Investigator to be serious and/or unexpected the Investigator must report it to the IRB/EC as soon as possible.

3M reports (by telephone or fax) a death or life-threatening adverse drug experience to the FDA within 7 days of notification, and submits a follow-up report within 15 days.

A serious and unexpected adverse drug experience involving a non-3M commercialized product is reported to the 3M study monitor and IRB/EC.

If a subject does not experience an AE during the study, the absence of such must be recorded on the form.

## **8. STATISTICS**

### **8.1 Data Sets Analyzed**

Subjects with an average treatment day baseline of  $\geq 3.0 \log_{10}$  CFU/cm<sup>2</sup> for the abdominal region and/or  $\geq 5.0 \log_{10}$  CFU/cm<sup>2</sup> for the inguinal region will be included in the analysis. The analyses will use paired t-tests. Thus, at any sample time, a subject must not only meet treatment day baseline requirements but also have  $\log_{10}$  recovery values from both products. At each sample time, analysis will be conducted using all subjects meeting treatment day baseline requirements and also having  $\log_{10}$  recovery values from both products at that sampling time. For the secondary outcomes, the subjects must also have  $\log_{10}$  recovery values from both products at 10 minutes.

### **8.2 Statistical Methods**

Paired t-tests will be used to analyze the primary and secondary outcomes by sampling time. A Hochberg step-up procedure will be used to adjust for multiple comparisons. This will be done separately for the primary outcomes and for the secondary outcomes. A family error rate of 0.05 will be used.

#### **8.2.1 Efficacy Analyses**

Summary tables will be produced for baseline  $\log_{10}$  recovery,  $\log_{10}$  reduction from baseline at 10 minutes,  $\log_{10}$  recovery at the 4 sampling times and  $\log_{10}$  regrowth from the 10 min  $\log_{10}$  recovery for 48 hours, 72 hours and 96 hours.

The mean paired difference between products, the 95% confidence interval and the p-value will be produced for  $\log_{10}$  recovery at 48 hours, 72 hours and 96 hours and for the  $\log_{10}$  regrowth from the 10 min  $\log_{10}$  recovery for 48 hours, 72 hours and 96 hours.

#### **8.2.2 Safety Analyses**

Adverse events and skin irritation scores will be summarized. McNemar's test will be used to test for a difference between products in adverse event.



### **8.3 Sample Size Justification**

The standard deviation of the paired differences in  $\log_{10}$  reduction observed in study [REDACTED] was, approximately, 1.0. A sample size of 45 provides 80% power to detect a difference of 0.5  $\log_{10}$  at  $\alpha=0.017$  (to allow for adjustment for multiple comparisons). Fifty subjects will be enrolled to allow for loss of subjects and uncertainty in variability.

### **8.4 Criteria for Termination of the Study**

3M reserves the right to terminate the study at any time for business reasons.

### **8.5 Procedures for Accounting for Missing, Unused, and Spurious Data**

Any non use of data will be discussed and justified in the blinded review of data at the data lock meeting and documented in the meeting minutes.

### **8.6 Deviations to Statistical Plan**

Any deviation from the statistical plan discussed above will be documented in the study report.

## **9. MONITORING**

3M, as sponsor of this study, is responsible for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded. 3M has therefore assigned a study monitor to this study. The progress of the study will be monitored by:

- Periodic on-site review
- Ongoing review of electronic documentation [REDACTED]
- Telephone and e-mail communications
- Review of source documents

The Investigator or designee will give the 3M study monitor direct access to source documents that support data on the eCRFs and make available such records to authorized 3M, quality assurance, IRB/EC, and regulatory personnel for inspection and/or copying.

## **10. QUALITY CONTROL AND QUALITY ASSURANCE**

3M is responsible for implementing and maintaining quality assurance and quality control systems through written standard operating procedures (SOPs) to ensure that this study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and regulations cited in Section 1.5 of this protocol. Study monitoring is carried out to accomplish this.

## **11. ETHICS**

This study will be conducted in accordance with the principles that have their origin in the Declaration of Helsinki, 21 CFR 50 (Informed Consent) and 56 (IRBs). The study will start only after approval of the protocol and consent form by the IRB/EC. The approval letter or notice must contain the IRB/EC name, meeting date, and sufficient information to identify the protocol

[REDACTED]

and informed consent by name and number that were reviewed. 3M, prior to study initiation, must receive a copy of the IRB/EC approval letter.

## **12. DATA HANDLING AND RECORD KEEPING**

### **12.1 Study Personnel**

Prior to study initiation, the Investigator must provide 3M with a signed Form FDA 1572. This form identifies all sub-investigators who will be assisting the Investigator in the conduct of the study. The Investigator will provide a log of signatures/initials prior to study initiation.

### **12.2 Pre-Study Documentation Requirements**

Prior to study initiation, the Investigator must provide 3M with the following documents:

- Signed protocol including any amendments in place prior to study initiation
- *Curriculum vitae* for the Investigator and any co-investigators
- IRB/EC approved consent form
- IRB/EC study approval letter
- IRB/EC name, location and membership list
- IRB/EC certification
- Signed Form FDA 1572
- Financial Disclosure
- Signed research agreement

### **12.3 Completion and Return of Case Report Forms**

Electronic data capture (eDC) software will be used for this study. Data may be recorded onto data collections sheets prior to data entry or may be entered directly into the eDC system. Microbiological count data will be recorded onto DCSs prior to entry into the eDC system. If a correction is required, a single line must be drawn through the error. The person making the correction will initial, date, and provide a reason for the error (if not self-evident). Once the forms are completed, the monitor will review the case report form (CRF) to assure accuracy and completeness.

### **12.4 Final Report**

[REDACTED]

The Investigator will submit the Investigator Final Report to 3M and the IRB/EC.

### **12.5 Records, Reports and Retention Requirements**

Study records must be kept until 3M provides notification that the documents can be destroyed. This may extend beyond the 2 years required by law. In order to comply with regulatory requirements, the Investigator must arrange for the retention of all study records for 2 years

[REDACTED]

following the date a marketing application is approved for the drug and indication investigated, or if no application is filed, until 2 years after completion/termination of the study [see 21 CFR 312.62 (c) and 21 CFR 812.140(d)].

Records to be retained in the Investigator study file include, but are not restricted to:

- Signed study protocol, amendments, deviations
- Applications to the IRB/EC
- IRB/EC approval of protocol, consent form, authorization form and amendments to any of these documents
- Signed consent forms
- Case report forms and/or data collection forms
- Adverse event reports
- Records of receipt, use, or disposition of the investigational drug
- Correspondence relating to the study
- Investigator Brochure
- Statement of Investigator
- Financial disclosure documents
- Final Report

### 13. REFERENCES

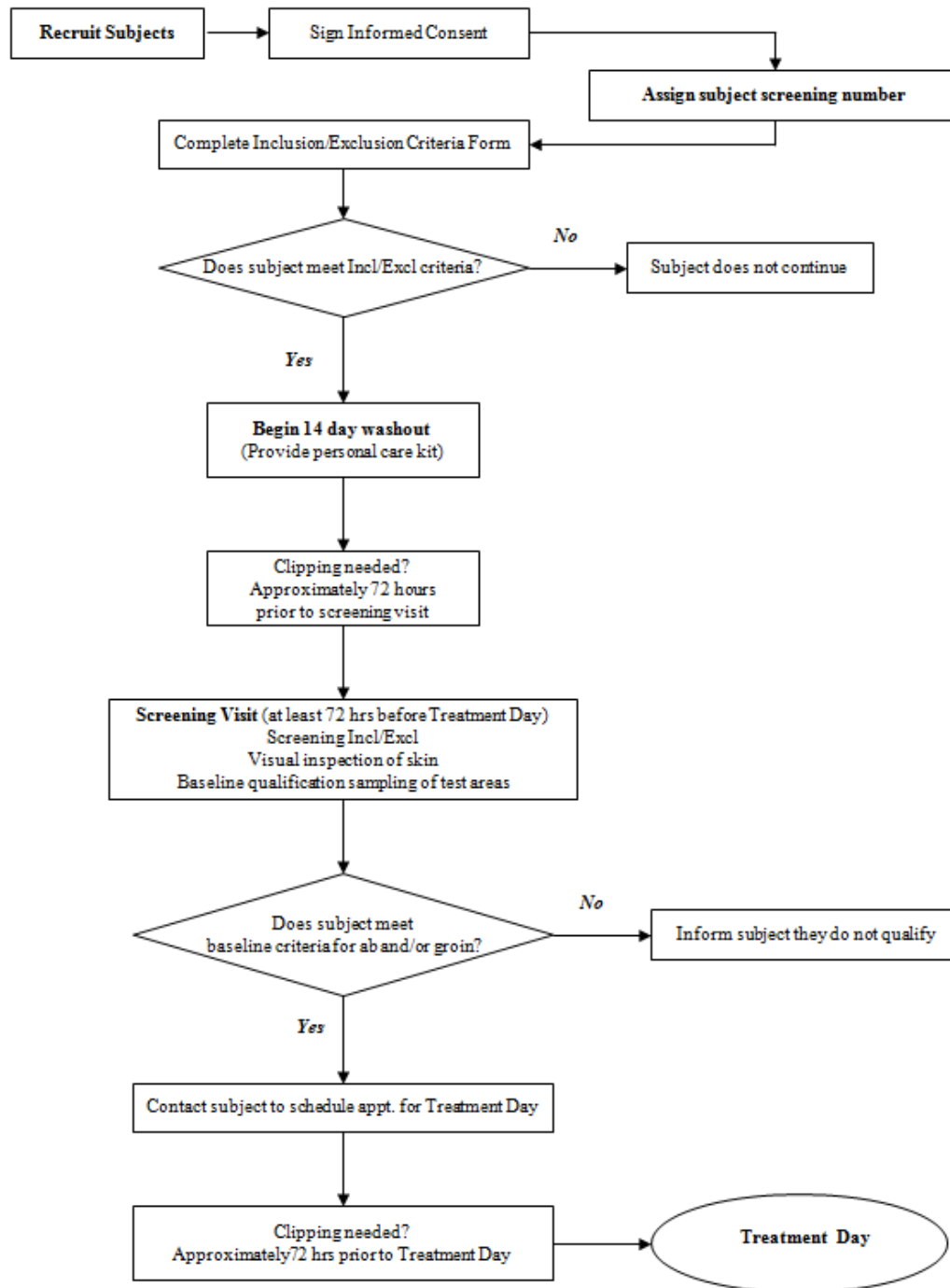
- 1 Butterfield, CT. The selection of a dilution water for bacteriological examinations. J Bacteriol 1932; 23: 355-68.
- 2 ASTM International. ASTM E1173, standard test method for evaluation of preoperative, precatheterization, or preinjection skin preparations. West Conshohocken [PA]: ASTM Int'l; 2015.
- 3 Williamson P, Kligman PM. A new method for the quantitative investigation of cutaneous bacteria. J Invest Dermatol 1965; 45:498-503.
- 4 ASTM International. ASTM E1054, standard test methods for evaluation of inactivators of antimicrobial agents. West Conshohocken [PA]: ASTM Int'l; 2013.



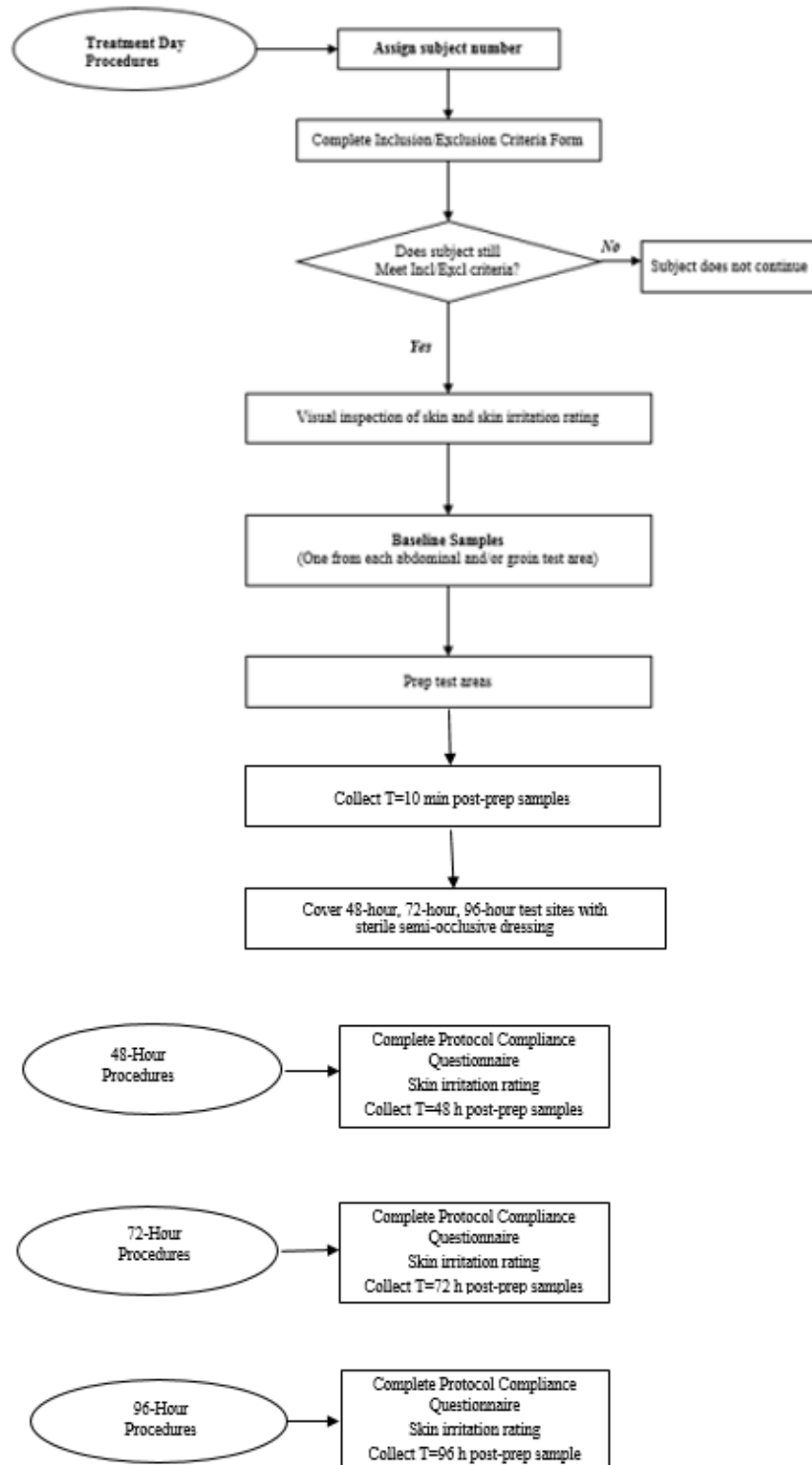
## 14. APPENDICES

### 14.1 Study Flow Diagrams

#### Screening Day



## Treatment Day



How often do you use the Internet?	Number of Responses
Never	2
Sometimes	4
Often	6
Very often	3

### 14.3 Confirmation of Release & Receipt of Clinical Supplies

[Redacted]			
[Redacted]			
[Redacted]	[Redacted]	[Redacted]	[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

[Redacted]

#### 14.4 Study Product Disposition Form

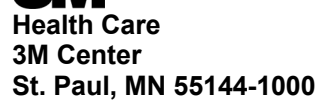
[REDACTED]

[REDACTED]	
[REDACTED]	
[REDACTED]	[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

[REDACTED]



## 14.5 Subject Instructions

[illegible]

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Frequency	Response Count
Never	1
Rarely	1
Sometimes	1
Often	1
Very often	1

## 14.6 Elements of Informed Consent

These elements of consent should be included as applicable to the study being conducted.

1. Statement that the study involves research.
2. Purpose(s) of the research.
3. Expected duration of subject's participation.
4. Procedures to be followed and identification of any procedures that are experimental.
5. A description of any reasonable foreseeable risks or discomforts to the subject.
  - a) Risks/discomforts from study procedures.
  - b) Foreseeable risks associated with the investigational product, which include adverse experiences listed in the Investigator's Brochure or package insert.
6. A description of any benefits to the subject or to others which may reasonably be expected from the research.
7. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.
8. Extent to which confidentiality of records identifying subject will be maintained.
  - a) Possibility that representatives of 3M and the FDA may inspect and make copies of the records.
  - b) Suggested text: "I understand that, at any time, an agent of 3M may also review any hospital, physician, or insurance billing or any other costs which relate to therapy incurred as a direct result of my participating in this study."
9. An explanation as to whether any compensation or medical treatments are available if injury occurs for research involving more than minimal risk. The explanation should involve a description of the compensation or treatment available or a statement describing where further information may be obtained.
10. Whom to contact for answers to pertinent questions about research and research subject's rights.
11. Whom to contact in the event of research-related injury to the subject.
12. Participation is voluntary:
  - a) Refusal to participate will involve no penalty or loss of benefits to which subject is otherwise entitled.
  - b) Subject may discontinue participation at any time without penalty or loss of benefit to which subject is otherwise entitled.



## ADDITIONAL ELEMENTS OF CONSENT

When appropriate, one or more of the following elements of information shall also be provided to each subject.

1. A statement that the particular treatment or procedure may involve risks to the subject (or embryo or fetus, if subject became pregnant) which are currently unforeseeable.
2. Anticipated circumstances under which subject's participation may be terminated by the Investigator without regard to subject's consent.
3. Any additional costs to the subject that may result from participation in the research.
4. A statement explaining the consequences of subject's decision to withdraw during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
5. A statement that significant new findings developed during the course of the research which may relate to subject's willingness to continue participation will be provided to the subject.
6. Approximate number of subjects involved in the study.

Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable federal, state, or local laws.

Informed consent allows the subject to fully understand his/her participation and serves to protect the Investigator and Sponsor from potential negligence claims. A fully informed subject is the best protection against such claims.

The informed consent requirements in these regulations are not intended to preempt any applicable federal, state, or local laws that require additional information be disclosed for informed consent to be legally effective. Some states, such as California and Oregon, require further action on the Investigator's part concerning subject consent.



[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

## 14.8 Skin Irritation Rating Scale


## 14.9 Protocol for Neutralization Validation

### 14.9.1 Background

In order to accurately assess the efficacy of an antimicrobial product, it is necessary to completely inactivate the antimicrobial agent at the time point being evaluated. Inadequate neutralization will allow killing or inhibition of the microorganisms to continue beyond the specified contact time, resulting in an overestimation of antimicrobial activity.

Standard sampling solution (SSS) is a buffered detergent solution that is commonly used in studies where microbial sampling of skin is conducted. Neutralizers have been added to inactivate the antimicrobial, CHG, present in the active study products. [REDACTED]

[REDACTED] The effectiveness and nontoxicity of these neutralizer systems must be assessed to demonstrate that there is no effect on the growth of microorganisms and that the antimicrobial ingredient is inactivated.

The density of normal human skin flora generally ranges from  $10^2$  to  $10^5$  CFU/cm<sup>2</sup> depending on the body site. However, since significant neutralizer or toxic effects are more easily detected at a lower cell density, the efficacy and toxicity of these neutralizers will be assessed against a lower bacterial concentration.

This is an *in vivo* test where the active study products are applied to the skin using the same application instructions used for the study (Appendix 14.2). Scrub cup samples are collected and inoculated with low levels of [REDACTED]

[REDACTED]. Two contact times after inoculation will be evaluated: immediately (<1 minute) and [REDACTED] (to test for residual antimicrobial activity between sample collection and the time of plating).

### 14.9.2 Objective

The objective of this assay is to determine the ability of the sampling solutions to completely neutralize the active ingredients contained in 3M CHG/IPA Prep and [REDACTED] when applied to the abdomen of test subjects without exhibiting toxicity to the test organisms.

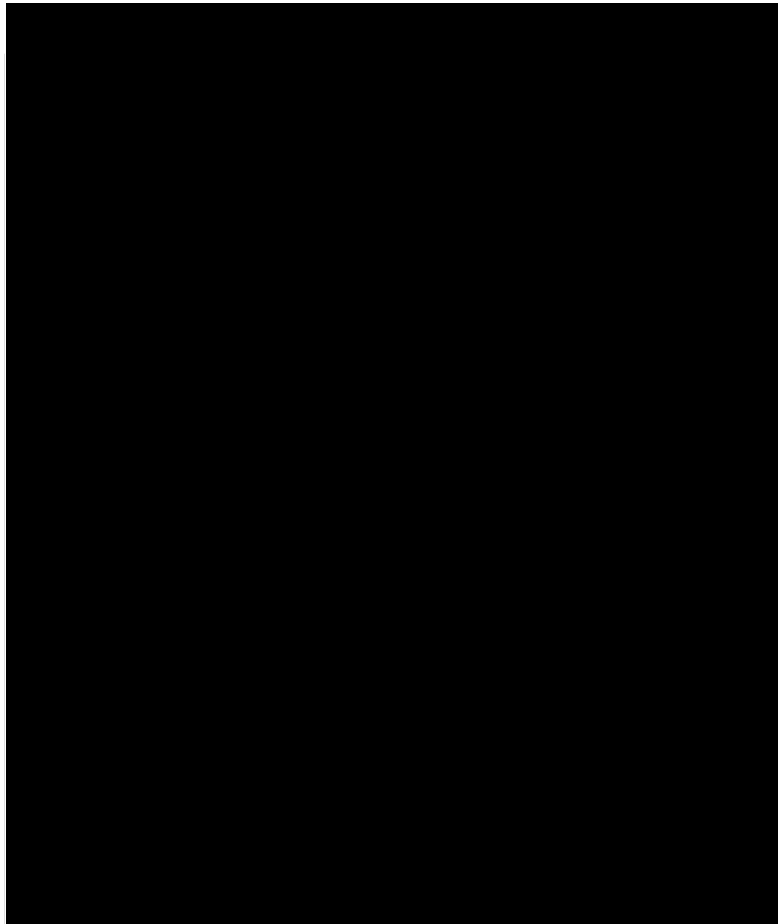
### 14.9.3 Subject Entry Criteria

Six subjects will participate in this study. Subjects must meet the inclusion and exclusion criteria in Sections 4.1 and 4.2 of the protocol to which this neutralizer validation is attached, except for the baseline bacterial count, the 72-hour exclusion from showering/bathing, and the length of the washout period. The neutralization subjects do not require a minimum baseline count and they only need to avoid topical and systemic antimicrobials for 7 days (not 14 days) prior to Treatment Day. Subjects will be asked to provide information on demographics and inclusion/exclusion criteria and sign the Informed Consent Form before beginning the 7-day washout period. When subjects return to begin their participation in the study they will again be asked to provide information relative to inclusion/exclusion criteria. If they meet all

[REDACTED]

inclusion/exclusion criteria, they may be enrolled. [REDACTED]  
[REDACTED]

Each subject will receive both active study products (3M CHG/IPA Prep and [REDACTED]), which will be applied to 2 separate test areas on the abdomen. See diagram below.



#### 14.9.4 Test Organism

The test organisms for this study are [REDACTED]  
[REDACTED]

#### 14.9.5 Study Products

- 3M CHG/IPA Prep, [REDACTED]
- [REDACTED]



- [illegible]

Frequency	Count
Never	1
Rarely	2
Sometimes	3
Often	4
Very often	3

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

#### **14.9.7 Study Procedures**

##### **14.9.7.1 Preparation of Inoculum**

One day prior to beginning the neutralization assay, the test organisms from a stock culture slant or from a lyophilized vial will be inoculated on TSA. The plates will be incubated for  $24 \pm 4$  hours at  $35^\circ \pm 2^\circ\text{C}$ .

Immediately prior to initiating the neutralization assay, an inoculum suspension for each test organism will be prepared in PBW solution from the overnight culture on the TSA plate, and the concentration adjusted to approximately  $10^8 - 10^9$  CFU/mL.

Dilute the inoculum suspension with PBW to achieve an appropriate concentration (approximately  $10^4$  CFU/mL) for inoculation of the test samples. The density of the test inocula must be verified by direct plating at the beginning and end of the study.

##### **14.9.7.2 Preparation of Test Area and Post-Prep Sampling**

Neutralization samples will be evaluated on the abdomen. The test areas on each subject's abdomen will be assigned according to a computer-generated randomization schedule to receive 1 of the 2 study products. The subject number, location of the product application, location of the sites sampled within the test area, and the time of sample collection will be documented. Each subject will be treated with both study products, one in each test area.

Abdominal test areas will be marked using a sterile 1.5" x 5" template [REDACTED]. One 1.5" x 5" area will be delineated on one side of the body containing two 1" x 1" sampling sites and the other 1.5" x 5" area will be delineated on the opposite side of the abdomen, containing two 1" x 1" sampling sites, to yield 4 sampling sites for each subject. One tube from each test area will be inoculated with [REDACTED] and the other will be inoculated with [REDACTED].

After the test areas are marked, each area will be prepped with three 70% isopropyl alcohol swabs for a total of 1 minute and allowed to dry for 3 minutes.

Each test area will be treated with 1 of the 2 study products, according to the instructions for the inguinal region provided in Appendix 14.2 of the protocol to which this neutralizer validation is attached.

Samples will be collected, using the scrub cup technique, at 10 minutes [REDACTED] post-prep. Post-prep timing begins upon completion of study product application, including the 3-minute drying time. The sampling technique is described in Section 6.2.1.1 of the protocol to which this neutralizer validation is attached.

[REDACTED]

Residual product should be removed from test areas after sample collection is complete [REDACTED]

Each pooled sample (~6 mL) will be capped tightly [REDACTED] followed by vortex mixing for 30 seconds. Five mL will be transferred to a clean tube and immediately inoculated with 100 µL of the appropriate diluted inoculum (Section 14.9.7.1 of the neutralization validation). This will yield an organism concentration of ~20 – 200 CFU/mL.

Immediately (<1 minute) and at [REDACTED] minutes [REDACTED] post-inoculation, [REDACTED] aliquots of the inoculated samples will be pour-plated in triplicate using TSA+N. Plates will be incubated inverted at  $35 \pm 2^{\circ}\text{C}$  for 48 [REDACTED] hours.

#### 14.9.7.3 Numbers Control and Toxicity Control

This testing will be performed in triplicate.

##### Numbers Control (NC):

Add 100 µL of the appropriate diluted inoculum to a tube containing 5 mL PBW to yield final inoculum concentrations of ~20-200 CFU/mL. [REDACTED]

[REDACTED] Pour-plate triplicate [REDACTED] aliquots immediately (<1 minute) and [REDACTED] minutes post-inoculation in the same manner as 14.9.7.2, except using TSA without neutralizers.

##### Toxicity Control (TC-MSS):

Add [REDACTED] 5.0 mL SS [REDACTED] to a clean tube. Add 100 µL of the appropriate diluted inoculum to the 5-mL tube to yield final inoculum concentrations of ~20-200 CFU/mL. Pour-plate triplicate [REDACTED] L aliquots immediately (<1 minute) and [REDACTED] post-inoculation in the same manner as 14.9.7.2.

These controls will provide assurance that the test organisms are not adversely affected by the treatments or the sampling procedures.

#### 14.9.7.4 Enumeration

[REDACTED]. Enumerate plates and calculate CFU/mL for each sample. Convert data to  $\log_{10}$  CFU/mL.

#### 14.9.8 Control of Bias

Bias is controlled by internal controls and triplicate plating.

#### 14.9.9 Data Evaluation

Recovery for each of the samples will be expressed as  $\log_{10}$  CFU/mL.



Neutralizer Effectiveness:

If the mean  $\log_{10}$  CFU/mL of the sample is not more than 0.3  $\log_{10}$  less than the mean  $\log_{10}$  CFU/mL of the NC, the neutralization will be considered effective.

- Mean  $\log_{10}$  CFU/mL from NC – Mean  $\log_{10}$  CFU/mL from sample (use corresponding time points)

Neutralizer Toxicity:

If the mean  $\log_{10}$  CFU/mL of the TC is not more than 0.3  $\log_{10}$  less than the mean  $\log_{10}$  CFU/mL of the NC, the sampling solutions will be considered non-toxic.

- Mean  $\log_{10}$  CFU/mL from NC – Mean  $\log_{10}$  CFU/mL from TC (use corresponding time points).

